The emergence of dyslexia in the developing brain

Ulrike Kuhl a,*, Nicole E. Neef a, Indra Kraft a, Gesa Schaadt b, Liane Dörr a, Jens Brauer a, Ivonne Czepezauer b, Bent Müller b, Arndt Wilcke b, Holger Kirsten b, Frank Emmrich b, Johannes Boltze b, c, Angela D. Friederici a, Michael A. Skeide a

a Department of Neuropsychology, Max Planck Institute for Human Cognitive and Brain Sciences, Stephanstr. 1a, 04103, Leipzig, Germany
b Fraunhofer Institute for Cell Therapy and Immunology, Perlickstr. 1, 04103, Leipzig, Germany
c Fraunhofer Institute for Cell Therapy and Immunology, Perlickstr. 1, 04103, Leipzig, Germany

ABSTRACT
Developmental dyslexia, a severe deficit in literacy learning, is a neurodevelopmental learning disorder. Yet, it is not clear whether existing neurobiological accounts of dyslexia capture potential predispositions of the deficit or consequences of reduced reading experience. Here, we longitudinally followed 32 children from preliterate to school age using functional and structural magnetic resonance imaging techniques. Based on standardised and age-normed reading and spelling tests administered at school age, children were classified as 16 dyslexic participants and 16 controls. This longitudinal design allowed us to disentangle possible neurobiological predispositions for developing dyslexia from effects of individual differences in literacy experience. In our sample, the disorder can be predicted already before literacy learning from auditory cortex gyriteraits in dyslexic individuals are at least in part similar to those observed in illiterates who have no practice in reading. Hence, discriminating potentially predisposing factors from consequences of DD is a major challenge for identifying neural correlates of dyslexia.

ARTICLE INFO
Keywords:
Developmental dyslexia
Developmental learning disorder
Reading development
Developmental cognitive neuroscience

1. Introduction
Developmental dyslexia (DD) is a specific neurodevelopmental learning disorder. Despite normal intellectual skills, affected individuals struggle severely with literacy acquisition, thus facing profound educational disadvantages throughout life. Impairments may affect reading and spelling specifically, and may occur independently of performance in other domains like mathematics (Skeide et al., 2018b). Beyond academic achievement, living with DD has considerable negative effects on mental health, such as an increased risk of anxiety disorders and depression, posing a costly burden on society (Klassen et al., 2013).

While speech processing deficits are most consistently found in dyslexic individuals, DD is considered to be a heterogeneous condition that may also relate to other domains, such as vision or attention. Accordingly, affected individuals show different cognitive profiles across these domains (Heim et al., 2008; Ramus et al., 2003; Steinbrink et al., 2014). Accordingly, there are various accounts aiming to explain the neurobiological underpinnings of DD (Table 1). These accounts, however, are largely based on data of school-aged or adult participants. This is a limitation, since differences between dyslexic participants and controls might also reflect confounding differences in literacy acquisition. In a recent study, Haft et al. (2018) demonstrated that children with dyslexia actively avoid reading-related stimuli. This bias may reduce reading practice of affected individuals compared to typically developing individuals. As a consequence, dyslexic individuals may vary in amount and quality of neuroplastic changes related to successful reading acquisition (Brem et al., 2010; Dehaene et al., 2015; Skeide et al., 2017). This line of argumentation has been recently adopted by Huettig et al. (2018), suggesting that cognitive deficits in dyslexic individuals are at least in part similar to those observed in illiterates who have no practice in reading. Hence, discriminating potentially predisposing factors from consequences of DD is a major challenge for identifying neural correlates of dyslexia.

Twin and family studies indicate an increased risk of literacy impairments in individuals with a dyslexic parent or sibling (DeFries et al., 1987; Lyytinen et al., 2004). Therefore, the question for possible predisposing neurobiological factors for DD has been extensively studied based on work in children with a family history of dyslexia. Magnetic resonance imaging (MRI) data suggest that preliterate children at a familial risk exhibit reduced grey matter volume and atypical cortical folding in left occipitotemporal and temporoparietal regions associated with reading (Im et al., 2016; Raschle et al., 2011). These structural
Table 1 Neurobiological accounts of dyslexia.

<table>
<thead>
<tr>
<th>Account</th>
<th>Potential core deficit</th>
<th>Target regions</th>
<th>Target tracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual deficit</td>
<td>decreased contrast</td>
<td>LGN (Livingstone et al., 1991)</td>
<td>LGN → V1</td>
</tr>
<tr>
<td></td>
<td>sensitivity caused by</td>
<td>VI (Livingstone et al., 1991)</td>
<td>MT → V1</td>
</tr>
<tr>
<td></td>
<td>reduced size and thus</td>
<td>MT (Eiden et al., 1996)</td>
<td>FG</td>
</tr>
<tr>
<td></td>
<td>faulty response of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>visual magnocells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auditory deficit</td>
<td>faulty perception of</td>
<td>IC (Hornickel and Kraus, 2013)</td>
<td>IC → MGB</td>
</tr>
<tr>
<td></td>
<td>short or rapidly</td>
<td>MGB (Galaburda et al., 1994)</td>
<td>MGB → AI</td>
</tr>
<tr>
<td></td>
<td>changing sounds due</td>
<td>AI (Clark et al., 2014; Leborgne et al., 2011)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>to decreased size of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>auditory magnocells</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and more variable</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>responses to</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>auditory stimuli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellar deficit</td>
<td>cerebellar hypoactivation, resulting in procedural sequence learning difficulties</td>
<td>Cerebellum (Freedman et al., 2017; Nicolson et al., 2001)</td>
<td></td>
</tr>
<tr>
<td>Phonological deficit</td>
<td>impaired representation and processing of speech sounds</td>
<td>A1, PT (Leborgne et al., 2011; Vandremerstenn et al., 2020)</td>
<td>A1 → PT</td>
</tr>
<tr>
<td>Orthographic deficit</td>
<td>deficient processing of print caused by reduced activation of neurons selectively tuned to printed words</td>
<td>FG (Cao et al., 2018; Salmelin et al., 1996)</td>
<td>FG → BA45/</td>
</tr>
</tbody>
</table>

BA—Brodmann area; LGN—lateral geniculate nucleus; VI—primary visual cortex; MT—middle temporal visual area; IC—inferior colliculus; MGB—medial geniculate nucleus; A1—primary auditory cortex; PT—planum temporale; FG—fusiform gyrus.

results are complemented by findings of reduced activity within bilateral occipitotemporal and left temporoparietal areas during phonological processing (Raschle et al., 2012). Similar effects have been reported for children that were diagnosed with dyslexia after formal literacy instruction (Williams et al., 2018). Beyond cortical anomalies, white matter differences within the left arcuate fasciculus connecting temporoparietal regions with premotor and inferior frontal areas were detected within children at a familial risk of developing dyslexia (Langer et al., 2017). Furthermore, these brain networks are also related to the strongest behavioural predictors of literacy outcome, namely phonological awareness and phonological short-term memory (Raschle et al., 2011; Saygin et al., 2013). Taken together, these results suggest that functional and structural alterations related to the emergence of DD may develop in early childhood and are thus already detectable before literacy onset.

Studies assessing preliterate children with a familial risk cross-sectionally do not allow to answer the question whether reported differences reflect potential predisposing factors of developing DD or consequences of reduced literacy exposition. Thus, longitudinal designs are crucial to validate insights derived from cross-sectional studies. There is first longitudinal evidence that infants who later developed dyslexia show reduced electrophysiological responses to speech (e.g., discriminating consonant onsets in the syllables /da/ vs. /ga/) already 2–5 months after birth (Schaadt et al., 2015; van Zuijen et al., 2013). Moreover, longitudinal structural MRI work revealed reduced thickness in left temporal and parietal cortices (Clark et al., 2014; Kraft et al., 2015), as well as reduced myelination of the arcuate fasciculus in future dyslexic children (Kraft et al., 2016; Vanderauwera et al., 2017). Recently, Beelen et al. (2019) identified anatomical anomalies in bilateral fusiform gyrus related to phonological awareness in pre-reading children who later developed dyslexia.

While the currently available data point to atypical functioning and maturation of the brain’s speech system, it is unclear whether differences in other sensory or cognitive domains highlighted in the literature (Table 1) already exist prior to literacy acquisition. Accordingly, the present study was designed to shed light on this topic. To this end, we followed children longitudinally that underwent multimodal MRI and psychometric testing before literacy instruction (at 5–6 years in kindergarten without pre-school education as typical in Germany) and after literacy instruction in school (at 7–8 years in second grade). Combining resting-state functional MRI (fMRI), T1-and diffusion-weighted imaging, we were able to comprehensively reconstruct the complex cortical and subcortical networks that were previously linked to DD. Building on this dataset, we examined functional and structural measures both before and after literacy instruction has begun, while controlling for sociodemographic factors (maternal education), domain-general cognitive capacities (non-verbal IQ, attention), and comorbid learning disorders (mathematical deficits). This approach allowed us to investigate whether dyslexia-related differences reported in older cohorts already existed in our cohort prior to literacy acquisition. To this end, we compared brain structural and functional measures between future dyslexic children and controls, separately for both measurement time points.

Following the current literature on preliterate children, we expected neural differences between dyslexic participants and controls to be confined to the speech system. In particular, we assumed that we were able to replicate previous results indicating atypical functioning and structural organisation of left auditory, superior temporal and inferior frontal cortices as well as the interconnecting arcuate fasciculus. Thus far, positive evidence for the persistence of these effects from preliteracy to literacy only exists for reduced cortical thickness of the left auditory cortex (Clark et al., 2014; but see methodological concerns expressed in Kraft et al., 2015). However, other morphometric indices that have not yet been investigated might also play a role. For instance, inherited polymicrogyria were reported in the left auditory cortex and the left superior temporal cortex in post-mortem investigations of dyslexic adults (Galaburda et al., 2006). Such malformations might rather be captured by indices quantifying gyriﬁcation, as suggested by previous cross-sectional work on children with dyslexia (Caverzasi et al., 2018). Accordingly, we tested the hypothesis that signiﬁcantly reduced cortical thickness and/or increased gyriﬁcation of the left auditory and/or the left superior temporal cortex distinguishes individuals with DD from controls both before and after literacy. Additionally, we expected group differences in functional and structural connectivity of auditory and superior temporal areas with downstream regions such as the premotor and inferior frontal cortex.

2. Methods and materials

Participants. Between 2012 and 2013, 82 children were recruited from the Leipzig metropolitan area for this study. To maximize the number of dyslexic participants in the final sample, individuals with a familial risk of developing dyslexia were the main target group of the recruitment procedure. 37 of the 82 children had at least one first- or second degree relative with DD. Status of familial risk of dyslexia was assessed as part of a questionnaire answered by the parents. All parents gave written informed consent and all children gave verbal informed assent to participate. The study was approved by the Ethics Committee of the University of Leipzig, Germany. Initial data acquisition took place between 2012 and 2013. Follow-up sessions were conducted between 2015 and 2016.

Initial screening ensured that participants did not have a history of neurological, psychiatric, hearing or vision disorders, that they were native German monolingual speakers and that they did not yet receive literacy training. In this context, it has to be noted that in many European
countries, including Germany, typical state kindergartens are not part of
the school system and do not provide formal literacy education.
Accordingly, while children have access to books that are read to them by
kindergarten caregivers, they typically do not train letter decoding before
entering school.

Further, 39 individuals were excluded from analysis because they
either received a diagnosis of attention deficit hyperactivity disorder
(ADHD, n = 4, determined based on parental questionnaire), exhibited
excessive head motion during functional and/or structural MRI (n = 20),
were unable to attend follow-up sessions (n = 4), did not finish all psy-
chometric tests (n = 7; Table 2) or performed below the 16th percentile
in a standardised math test (n = 4, Table 2, to exclude participants with
developmental dyscalculia). Moreover, one additional participant had to
be excluded due to an experimental error during psychometric testing.
A follow-up assessment based on a parental questionnaire ruled out that
children in our sample were diagnosed with ADHD between kindergarten
and the end of second grade in school.

In the remaining sample of 42 children, dyslexia was operationally
defined at the end of second grade based on standardised and age-
normed reading and spelling tests (Table 2). Performance below the
16th percentile rank (equivalent to 1 standard deviation below the mean
of the normal distribution or a t-score of 40) of the test’s reference
population performance in either one or both spelling accuracy and real-
word reading fluency led to assignment to the dyslexic group. Our final
sample included 3 children with isolated spelling deficits, 3 children with
isolated reading deficits, and 10 children with combined deficits. For our
control group, we only considered children that had neither first- nor
second-degree relatives with DD (n = 21; assessed by parental
questionnaire). Further, to exclude poor readers and spellers that did not
meet the criterion for being considered dyslexic, only individuals per-
foming above the 25th percentile rank were assigned to the control
group. Consequently, n = 5 poor readers or spellers scoring between the
16th and the 25th percentile rank were excluded to form distinct groups
and to avoid performance overlap (Shaywitz et al., 2002). Applying these
criteria, 16 children were classified as dyslexic and 16 children were
classified as typically developing controls following our operational
definition (Table 3). None of the participants in the final sample scored
below 85 (equivalent to the 16th percentile rank, 1 standard deviation
below the mean of the normal distribution and a t-score of 40) on average
in the two non-verbal IQ tests.

Additionally, two controls had to be excluded from the resting-state
fMRI analysis of kindergarten data, and two dyslexic participants were
excluded from the functional data analysis of school-age data due to
excessive head motion during the respective scans.

MRI data acquisition. MRI data were acquired on a 3T Trio scanner
(Siemens, Erlangen, Germany) at a preliterate age (kindergarten) and on
the same scanner upgraded to a 3T Prisma (Siemens, Erlangen, Germany)
at the end of second grade (Table 4).

T1-weighted data processing. T1-weighted MP2RAGE images were first
visually inspected to exclude data corrupted by imaging artefacts
including diffuse image noise along the phase-encoding direction,
ghosting or Gibbs artefact. Subsequently, images were skull-stripped
(Freesurfer, Version 5.3.0, http://surfer.nmr.mgh.harvard.edu/) and
used to create a group template with an isotropic resolution of 1.0 mm
in Montreal Neurological Institute (MNI) space (Advanced Normalization
Tools, Version 2.2.0, http://picasl.upenn.edu/software/ants/) to which
individual images were normalised (Computational Anatomy Toolbox,
CAT12, Version r1109; http://www.neuro.uni-jena.de/cat/).

Furthermore, T1 data were segmented into grey and white matter (CAT12,
SPM12, Update Revision Number 6906, http://fsl.fmrib.ox.ac.uk/)
in MATLAB R2017b, The Mathworks, Inc., Natick, MA, USA). Finally, maps
of cortical thickness (CT), gyriﬁcation index (GI), cortical folding
complexity (CF), and sulcus depth (SD) were extracted for each partici-
 pant and smoothed with a 15 mm full width at half maximum (FWHM)
kernel, in accordance with the matched-filter theorem. T1 images were
also extracted, but not smoothed.

ROI selection. Cortical, participant-speciﬁc region of interest masks
(ROIs, Table 5) were obtained by aligning a multi-modal parcellation of
brain areas comprising 180 cortical regions per hemisphere (Glasser
et al., 2016, retrieved 09/01/2016 from https://balsa.wustl.edu/study/
show/RVVG) to each participant’s MNI-T1 image and to Freesurfer’s
fsvaverage image. Additionally, subcortical areas MGB, LGN, and IC were
manually deﬁned by two of the authors (U.K., M.A.S.), independently
from each other on a T1 template with a resolution of 0.5mm3 isotropic
(kernel retrieved 09/05/2016 from http://opensciences.cbs.mpg.de/bazin/T
T_Quantitative/Group_Atlases/). The overlap of both definitions was
taken as ﬁnal subcortical ROI. Location of thalamic ROIs corresponds to
coordinates speciﬁed in the literature from other manual and
connectivity-based segmentations (Devlin et al., 2006). Finally, the
Spatially Unbiased Atlas Template (Diedrichsen, 2006; Diedrichsen et al.,
2009; Version 3.2, retrieved 09/05/2017 from http://www.diedrich
senlab.org/imaging/suit.htm) of cerebellum and brainstem were used to
extract a cerebellar ROI.

Diffusion-weighted data processing. Prior to pre-processing, diffusion-
weighted echo-planar imaging (EPI) MRI data were semi-automatically
and visually inspected for motion artefacts by identifying signal drop-
outs (Schreiber et al., 2014). Pre-processing was performed using FSL
v5.0 (https://fsl.fmrib.ox.ac.uk). Data were corrected for motion by
affinely aligning volumes with different b-values to respective averages
previously rigidly aligned with the individual participant’s MNI-T1
image.

Susceptibility artefacts were corrected based on two diffusion vol-
umes with reverse phase encoding (FSL TOPUP) additionally acquired for
each participant. To preserve high data quality, all transformations were

---

**Table 2**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Test</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kindergarten (before literacy, age 5–6)</td>
<td>Edinburgh Handedness Inventory (Oldfield, 1971)</td>
<td>laterality quotient</td>
</tr>
<tr>
<td>Hand edness</td>
<td>Self-constructed questionnaire</td>
<td>combined school and vocational qualification</td>
</tr>
<tr>
<td>Non-verbal intelligence</td>
<td>Wechsler preschool and primary scale of intelligence (WPPSI-III) (Wechsler et al., 2009)</td>
<td>performance IQ</td>
</tr>
<tr>
<td>Phonological awareness</td>
<td>Bielefeld screening of literacy precursor abilities (BISC) (Jansen et al., 1999)</td>
<td>composite score from rhyming, sound association, syllable segmentation, sound-to-word matching tasks</td>
</tr>
<tr>
<td>Rapid naming</td>
<td>BISC (Jansen et al., 1999)</td>
<td>rapid naming of coloured objects</td>
</tr>
<tr>
<td>Phonological short-term</td>
<td>Kaufman Assessment Battery for Children (K-ABC) (Kaufman et al., 2009)</td>
<td>recall of verbally presented number sequences of ascending length</td>
</tr>
<tr>
<td>Second grade (age 8–9)</td>
<td>Spelling accuracy</td>
<td>writing from dictation</td>
</tr>
<tr>
<td></td>
<td>German spelling test (DEREIT-2) (Stock and Schnitzler, 2008)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reading fluency</td>
<td>real word reading speed</td>
</tr>
<tr>
<td></td>
<td>Salzburg test of reading and spelling, second edition (SRT-II) (Moll and Landerl, 2010)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-verbal intelligence</td>
<td>perceptual reasoning IQ</td>
</tr>
<tr>
<td></td>
<td>Wechsler intelligence scale for children (WISC-IV) (Petermann and Petermann, 2011)</td>
<td></td>
</tr>
<tr>
<td>Mathematical ability</td>
<td>Heidelberg maths test (HRT-1-4) (Jaffuer et al., 2005)</td>
<td>composite score of arithmetic performance and numerical-logical reasoning</td>
</tr>
<tr>
<td>Phonological awareness</td>
<td>Basic competences for reading and writing abilities (BAKO) (Stock et al., 2003)</td>
<td>composite score of pseudo word segmentation, vowel-replacement, word completion, phoneme exchange, sound categorization, vowel length judgment, word reversal tasks</td>
</tr>
</tbody>
</table>
Table 3
Demographic information and psychometric performance.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Before literacy instruction</th>
<th>Dyslexia</th>
<th>Control</th>
<th>statistic</th>
<th>After literacy instruction</th>
<th>Dyslexia</th>
<th>Control</th>
<th>statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td>16</td>
<td>16</td>
<td>...</td>
<td></td>
<td>16</td>
<td>16</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>5±8 ±0 4</td>
<td>5±6 ±4</td>
<td>U-169</td>
<td></td>
<td>8±8 ±0 3</td>
<td>8±4 ±3</td>
<td>t(29)=−3.35; 0.0022&lt;</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>11/5</td>
<td>9/7</td>
<td>OR=−1.68&lt;</td>
<td></td>
<td>0.7160&lt;</td>
<td>0.5893&lt;</td>
<td>0.4344&lt;</td>
<td>0.051</td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
<td>3.94±0.98</td>
<td>4.63±1.15</td>
<td>U=−84</td>
<td></td>
<td>0.893&lt;</td>
<td>0.893&lt;</td>
<td>0.893&lt;</td>
<td>0.893&lt;</td>
</tr>
<tr>
<td>Handedness</td>
<td></td>
<td>79.06±15.22</td>
<td>65.38±39.63</td>
<td>U=−148.5</td>
<td></td>
<td>0.4344&lt;</td>
<td>0.4344&lt;</td>
<td>0.4344&lt;</td>
<td>0.4344&lt;</td>
</tr>
<tr>
<td>Familial risk status</td>
<td></td>
<td>10/6</td>
<td>0/16</td>
<td>...</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Psychometrics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-verbal IQ</td>
<td></td>
<td>99±1±12</td>
<td>109±1±2</td>
<td>t(29)=2.39; 0.0236&lt;</td>
<td></td>
<td>107±1±3</td>
<td>114±1±3</td>
<td>t(29)=1.66; 0.1081&lt;</td>
<td></td>
</tr>
<tr>
<td>Phonological short-term memory</td>
<td></td>
<td>9±1±93</td>
<td>10±25±1.77</td>
<td>U=179.5</td>
<td></td>
<td>0.0473&lt;</td>
<td>0.0368&lt;</td>
<td>0.0368&lt;</td>
<td>0.0368&lt;</td>
</tr>
<tr>
<td>Phonological awareness</td>
<td></td>
<td>32.16±4.72</td>
<td>35±38±5.59</td>
<td>t(28)=2.19; 0.0368&lt;</td>
<td></td>
<td>21.88±17.84; 62.63±20.78</td>
<td>U=236</td>
<td>&lt;0.0001&lt;</td>
<td></td>
</tr>
<tr>
<td>Rapid naming</td>
<td></td>
<td>6.06±2.02</td>
<td>6.81±1±05</td>
<td>U=162</td>
<td></td>
<td>0.1790&lt;</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Spelling accuracy</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Reading fluency</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
<td>15.19±15.52; 55.94±22.83</td>
<td>U=240</td>
<td>&lt;0.0001&lt;</td>
<td></td>
</tr>
</tbody>
</table>

*Years; months, age at MRI-scan, mean ± standard deviation (std).
Wilcoxon-Mann-Whitney U test (data not normally distributed).
Welch two-sample *t*-test (data normally distributed).
Fisher’s exact test.
Odd’s ratio.
F-test derived, combined score of mother’s school education (4-point scale: no degree: 0 points; German ‘Abitur’ [high school diploma/A level]: 3 points) and vocational qualification (5-point scale: no qualification: 0 points; German ‘Habilitation’ [postdoctoral academic qualification]: 4 points); mean±std.
L laterality quotient; scores range from −100 (left handed) to 100 (right handed), left-handedness is defined as an LQ<−28, i.e. the first decile vecity; right-handedness defined as LQ≥+28, i.e. the first decile vecity; ambidexterity: −28–laterality quotient<+28; mean±std.
Familial risk of DD/no familial risk of DD.
Average normed IQ score is 100 with a standard deviation of ±15; i.e.; mean±std.
Raw scores of number sequence recall task; sequence length increases every 3 items from 2 to maximally 9 until all three items of a length are recalled incorrectly; children receive a point for each correctly recalled number sequence; mean±std.
Before literacy instruction: combined number of correct responses (max. 40, 10 per task) from rhyming, sound association, syllable segmentation, and sound-to-word matching tasks; after literacy instruction: average standardised scores (percentile ranks) from pseudoword segmentation, vowel-replacement, word completion, phoneme exchange, sound categorization, vowel length judgment, and word reversal tasks; mean±std.
Time needed to rapidly name colours of 24 visually presented black and white objects, converted to scores ranging from 0 to 8; mean±std.
After writing dictation, standardised scores (percentile ranks) based on spelling accuracy (DERET-1); mean±std.
Reading speed, standardised scores (percentile ranks) based on number of real words correctly read within 1 min (SLRT-II); mean±std.

Table 4
MRI protocols.

<table>
<thead>
<tr>
<th></th>
<th>TI</th>
<th>dwMRI</th>
<th>rsMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Time point 1</td>
<td>Time point 2</td>
<td>Time point 1</td>
</tr>
<tr>
<td>TE (ms)</td>
<td>2.82</td>
<td>2.01</td>
<td>83</td>
</tr>
<tr>
<td>TR (ms)</td>
<td>5000</td>
<td>5000</td>
<td>8000</td>
</tr>
<tr>
<td>TI (ms)</td>
<td>700</td>
<td>700</td>
<td>700</td>
</tr>
<tr>
<td>Voxel size (mm x mm x mm)</td>
<td>1.3 x 1.0 x 1.9</td>
<td>1.7 x 3.0 x 3.0</td>
<td>1.3 x 1.0 x 1.9</td>
</tr>
<tr>
<td>FoV (mm x mm x mm)</td>
<td>250 x 256 x 186</td>
<td>210 x 192 x 192</td>
<td>219 x 240 x 186</td>
</tr>
<tr>
<td>No. of diffusion encoding directions</td>
<td>–</td>
<td>–</td>
<td>60</td>
</tr>
<tr>
<td>b-values (s/mm²)</td>
<td>–</td>
<td>–</td>
<td>1000</td>
</tr>
<tr>
<td>No. of b-values images</td>
<td>–</td>
<td>–</td>
<td>7</td>
</tr>
</tbody>
</table>

TI = T1-weighted scan; dwMRI = diffusion-weighted MRI; rsfMRI = resting-state functional MRI; TE = echo time; TR = repetition time; TI = inversion time; FoV = field of view.

combined and applied in a single step of interoplation. Finally, the diffusion tensor (FSL DTIFIT) and the fibre orientation distribution for each voxel were determined (FSL BEDPOSTX). Fractional anisotropy and mean diffusivity were derived from the tensor fit. Tractograms were computed by applying probabilistic tractography (FSL PROBTRACKX2). ROI masks of target tracts (Table 1) were generated by using each cortical

Table 5
Definition of cortical regions of interest derived from the Glasser atlas.

<table>
<thead>
<tr>
<th>Regions of interest</th>
<th>Atlas labels*</th>
</tr>
</thead>
<tbody>
<tr>
<td>left primary visual cortex (V1)</td>
<td>L_V1_ROI, 1</td>
</tr>
<tr>
<td>left middle temporal area (MT)</td>
<td>L_MT_ROI, 23</td>
</tr>
<tr>
<td>left fusiform gyrus (FG)</td>
<td>L_VVCO ROI, 163</td>
</tr>
<tr>
<td>left primary auditory cortex (A1)</td>
<td>L_A1_ROI, 24</td>
</tr>
<tr>
<td>left planum temporale (PT)</td>
<td>L_Pbel ROI, 174; L_Pbel ROI, 124</td>
</tr>
<tr>
<td>left ventral premotor cortex (BA6)</td>
<td>L_6r ROI, 56; L_6r ROI, 78</td>
</tr>
<tr>
<td>left pars opercularis of the inferior frontal gyrus (BA44)</td>
<td>L_44 ROI, 74; L_47 ROI, 76</td>
</tr>
<tr>
<td>left pars triangularis/orbitals of the inferior frontal gyrus (BA45/47)</td>
<td>L_45 ROI, 75; L_47 ROI, 76</td>
</tr>
</tbody>
</table>

BA=Brodman area; *Glasser et al. (2016), retrieved 09/01/2016 from https://baha.wustl.edu/study/show/RVVC; if several areas are given, they were combined to form the final region of interest.

ROI involved, once as seed region and once as target region. We seeded 5000 streamlines (curvature threshold = 0.2, step length = 0.5 mm) in each voxel within the grey matter-white matter interface of the seed region at hand. While this approach produced confined tracts for most of the target connections of interest (Table 1), tracking was restricted using exclusion masks to quantify structural connectivity of the left planum temporale with inferior frontal regions BA6 and BA44 and local connectivity between the left primary auditory cortex and left planum temporale via the dorsally located arcuate fasciculus. To this end, we used a rectangular ventral exclusion mask for the tract passing through the planum temporale and BA6/BA44 and a combination of a ventral and dorsal exclusion mask for the tract passing through the auditory cortex.
and the planum temporale. The corresponding masks were defined within the common MNI group templates for the respective time points. Position in y-direction of the ventral exclusion mask was 5 mm anterior of the maximal y-coordinate of all participant’s planum temporale seeds, spanning 3 mm into the anterior direction (i.e., y = 7–9). In x- and z-direction, the plane extended from coordinate 0 into the negative directions (i.e., x = 0 to −61, z = 0 to −49), covering the entire anterior temporal lobe. The dorsal exclusion mask was defined as a plane covering the whole field of view in x- and y-direction for z = 22–24. Resulting streamline density maps were first log-transformed and then voxel-wise divided by the log-transformed maximal number of possible streamlines. Summed, log-transformed and normalised maps were averaged and thresholded at the 80th percentile to extract only the core of the respective tract.

Resting-state fMRI data processing. T2*-weighted gradient-echo EPI resting-state fMRI (rsfMRI) data were preprocessed using FSL v5.0, Matlab R2017b and AFNI Version 17.2.17 (https://afni.nimh.nih.gov/). After removal of the first 4 vol of each scan, data were slice time corrected. Head motion was quantified by frame-wise displacement (the sum of rotational and translational rigid body realignment parameters from one volume to the next) (Power et al., 2012). To account for head motion, volumes with frame-wise displacement >0.5 mm were excluded from further analysis, yielding 75 vol per participant at time 1 and 118 vol per participant at time 2. Due to excessive head motion, two controls were excluded from further rsfMRI analysis at time point 1 (i.e., N = 16 dyslexic, N = 14 controls). For the same reason, 2 dyslexic individuals had to be excluded from the rsfMRI analysis at time point 2 (i.e., N = 14 dyslexic participants, N = 16 controls). Partial volume maps for grey matter, white matter, and cerebrospinal fluid were generated from the segmented MNI-T1 data (FSL FAST). White matter (WM) and cerebrospinal fluid (CSF) masks were thresholded at 80% tissue probability, before rigid alignment to individual rsfMRI space. To control for motion as well as scanner-related and physiological noise, five principal components from WM and CSF were regressed out together with the six linearly detrended motion parameters previously determined (Muschelli et al., 2014). Finally, residual data were bandpass-filtered at 0.01–0.1Hz and spatially smoothed with a 6 mm FWHM kernel, leading to an effective smoothness of around 8–9 mm given the resolution of 3.0 × 3.0 × 3.99 of the rsfMRI data. To investigate local as well as global similarity of blood oxygenation level dependent time-series (functional connectivity), we extracted and converted regional homogeneity and fractional amplitude of low frequency fluctuations from each grey matter voxel to z-scores (Yan and Zang, 2010). Mean functional connectivity was computed by extracting mean hemodynamic time-series for each ROI and calculating pair-wise correlations between areas that are connected via target tracts (i.e., the 10 connections shown in Table 1 and Supplementary Table 1) using AFNI 3dfim+.

Experimental Design and Statistical Analysis. Dyslexic and control participants underwent MRI (Table 4) and psychometric assessment (Table 2) at two time points, that is, once at age 5–6 in kindergarten (before they acquired literacy skills) and again approximately 2 years and 11 months later at the end of second grade (range of time between measurements: 2.2–3.8 years; children were age 8–9 at s time point).

In terms of psychometric testing (Table 2), we used measures quantifying spelling accuracy and real-word reading fluency at the end of second grade in school to operationally define DD. Further, measures of handwriting speed (number of words written per minute) were included as covariates in the statistical models assessing neural differences between dyslexic and control participants at the respective time points. Additionally, we covaried out maternal education (i.e., the combination of the mother’s highest educational and vocational qualification) which served as a proxy for home literacy environment (Rasheid et al., 2005).

Measures of literacy precursor abilities assessed at kindergarten age, including phonological awareness, rapid naming and phonological short-term memory, were used as behavioural predictors in a receiver operating characteristic curves (ROC) analysis. Lastly, re-assessment of phonological awareness at school age allowed us to see whether group differences with respect to this important literacy precursor ability still hold after two years of formal literacy instruction. The rationale behind this approach is that phonological awareness is one of the best known predictors of literacy skills but at the same time known to be modulated by reading experience (Peterson and Pennington, 2015). In line with this previous evidence, we were able to demonstrate that children who develop dyslexia do not only have lower phonological awareness compared to controls already before formal literacy instruction, but that their phonological awareness also does not significantly improve with instruction which is a hallmark feature of typical literacy learning (Table 3).

Demographic and behavioural data were tested for normality of distributions using the Shapiro-Wilk test. To compare groups, we used the non-parametric Wilcoxon-Mann-Whitney U test in case of non-normality, the Fisher’s exact test for nominal data, and the Welch two-sample t-test otherwise (all two-tailed).

For all 8 cortical ROIs (see Supplementary Table 1), means of CT, CF, GI, SD were extracted in MNI space. For all 8 cortical ROIs, and for LGN, MGB and the cerebellar ROI, we additionally extracted mean values of regional homogeneity (ReHo), fractional amplitude of low frequency fluctuations (fALFF), and structural T1-signal. Additionally, we quantified volumes of LGN and MGB masks for subsequent group analyses. Mean functional connectivity was computed by extracting mean haemodynamic time-series for each ROI and calculating pair-wise correlations between areas that are connected via target tracts (i.e., the 10 connections shown in Table 1 and Supplementary Table 1; AFNI 3dfim+). Fractional anisotropy (FA), mean diffusivity (MD), and streamline density (SLD) were computed voxel-wise along the 10 tracts identified by probabilistic tractography in SPM. ROI-wise or ROI-pair-wise comparisons of the different mean brain measures were performed using R–3.3.3 (https://www.r-project.org/) by running multiple one-way analyses of covariance conducted at each time point (i.e., kindergarten and primary school age) separately. Analyses included the covariates age, sex, handedness, and maternal education. Further, information about familial risk status was included in all statistical models to ensure that identified effects are related to literacy outcome rather than familial risk. Additionally, we included arithmetic ability as a covariate for all analyses in order to single out differences specific to literacy deficits, independent of individual mathematical ability. Finally, IQ was also included as a covariate for all analysis to remove the effect of general cognitive skills. We used IQ scores at time point 2 because IQ measures were shown to be more reliable in school-age than in preschool children (Bishop et al., 2003). To minimise the variance induced by these covariates we adjusted the analyses not only for covariates revealing significant group differences, but also for covariates revealing marginally significant and non-significant group differences (Table 3).

To ensure homogeneity of regression slopes, possible interactions between individual covariates and the categorical predictor variable were assessed beforehand. Homogeneity of regression curves is given for all comparisons despite a significant interaction between group and sex for functional connectivity between left primary auditory cortex and left planum temporale at time 1. Hence, separate analyses were run for male and female participants. Within each comparison, results were family-wise-error-corrected for the respective number of ROIs (see Supplementary Table 1). For comparison of surface-based measures (CT, GI, SD, CF) the critical α level was set to 0.0063 (corrected for all 8 cortical ROIs). For resting-state measures (fALFF and ReHo and the structural T1-signal, the critical α level was set to 0.0046 (corrected for 8 cortical, 2 subcortical and 1 cerebellar ROI). For the functional and structural connectivity analyses, the critical α value was set to 0.005 (i.e., accounting for 10 connections/tracts). To additionally test for potential effects not covered by our ROIs, we performed whole-brain analyses of each brain measure.

To investigate whether the brain data can significantly improve the
prediction of DD compared to behavioural data alone, we calculated receiver operating characteristic curves (ROC). Specifically, we computed binary logistic regression models to predict the relationship between our independent variables and dyslexia status. These models return conditional probabilities for belonging to the dyslexic group given the values of the respective observed predictor variables for each input sample. For each potential decision threshold separating the two groups, the proportion of actually positive samples out of all samples that were identified as being positive and the proportion of actually negative samples out of all samples that were identified as being negative are obtained. These correspond to the sensitivity and specificity, respectively, that make up the final ROC. Thus, the ROC and their corresponding areas under the curve (AUC) allow to evaluate the discriminatory power of the respective model. Variance inflation factor computed on all models indicated only weak multicollinearity between predictors (range 1.00–3.41). Areas under the receiver operating characteristic curve (AUC) of all models were compared using a two-tailed bootstrapping approach.

3. Results

Individuals with DD had significantly lower phonological processing skills compared to controls, both before literacy instruction at mean age 5y±7m (phonological short-term memory: \( N = 32, U = 179.5, p = 0.0473, d = 0.75, \) two-tailed; phonological awareness: \( N = 32, t(28) = 2.19, p = 0.0368, d = 0.78, \) two-tailed) and after literacy instruction at mean age 8y±6m (phonological awareness: \( N = 32, U = 236, p < 0.0001, d = 2.06, \) two-tailed) (Table 3). Moreover, literacy skills were significantly reduced in dyslexic individuals versus controls (\( N = 32, \) reading fluency: \( U = 238, p < 0.0001, d = 2.14, \) two-tailed; spelling accuracy: \( N = 32, U = 240, p < 0.0001, d = 2.23, \) two-tailed).

Group comparisons of the various cortical and subcortical measures (Fig. 1) revealed significantly higher gyriﬁcation of the left primary auditory cortex in dyslexic children compared to controls, persistent across time points (before literacy: \( N = 32, F(1,24) = 9.64, p = 0.0048, \eta^2 = 0.19; \) after literacy: \( N = 32, F(1,24) = 9.21, p = 0.0057, \eta^2 = 0.22). Additionally, functional connectivity between left primary auditory cortex and left planum temporale was signiﬁcantly lower in dyslexic children before literacy acquisition (\( N = 30, F(1,24) = 14.73, p = 0.0009, \eta^2 = 0.32). This effect was driven by a signiﬁcant difference in boys (\( N = 20, F(1,13) = 34.58, p = 0.0001, \eta^2 = 0.45), \) but not girls (\( N = 10, F(1,3) = 0.05, p = 0.8388, \eta^2 = 0.01). In terms of structural connectivity, we found signiﬁcantly higher streamline density at a preliterate age for dyslexic children compared to controls in the white matter fibres tract connecting the left planum temporale with the left ventral premotor area (BA 6), i.e., the arcuate fasciculus (\( N = 32, 70 \) voxels, \( F(1,24) = 19.80, p = 0.0040, \eta^2 = 0.45). No other region-of-interest and whole-brain control analysis revealed any additional statistically signiﬁcant effects for any neural indices.

Predictive sensitivity and speciﬁcity of the three signiﬁcant neural indices were compared against powerful behavioural predictors of literacy outcome known from the literature, namely phonological

![Fig. 1. Overview of signiﬁcant neural differences between dyslexic and control children. Horizontal lines within the bars represent the group median. Vertical lines at the top and the bottom of the bars depict the standard deviation. Red diamonds denote the mean of the distribution. Grey and black dots represent individual data points. Rotated kernel density plots on each side of the bar show the probability density of the data at different value. N = 32 for comparisons of structural measures, N = 30 for comparisons of functional connectivity. Asterisks indicate family-wise-error-corrected differences signiﬁcant at \( p < 0.05). A1 = primary auditory cortex; PT = planum temporale; BA6 = Brodmann Area 6; AF = arcuate fasciculus. In terms of structural connectivity, we found signiﬁcantly higher streamline density at a preliterate age for dyslexic children compared to controls in the white matter ﬁbre tract connecting the left planum temporale with the left ventral premotor area (BA 6), i.e., the arcuate fasciculus (\( N = 32, 70 \) voxels, \( F(1,24) = 19.80, p = 0.0040, \eta^2 = 0.45). All signiﬁcant statistics survived family-wise-error-correction for multiple comparison at the respective critical \( \alpha \) levels, i.e., \( \alpha = 0.0063 \) for comparison of surface-based measures (CT, GI, SD, CF); \( \alpha = 0.0046 \) for comparisons of fALFF, ReHo, T1-signal, tractwise mean MD, FA and SLD and seed-target based functional connectivity. Voxel-wise whole-brain analyses were assessed at a signiﬁcance level of \( \alpha < 0.05 \) (family-wise-error-corrected). No other region-of-interest and none of the whole-brain control analyses revealed any additional statistically signiﬁcant effects for any neural indices.](image-url)
awareness, and phonological short-term memory (Raschle et al., 2011; Saygin et al., 2013). Further, we included rapid naming ability as a measure prominently associated with reading outcome, especially for transparent orthographies (de Jong and van der Leij, 2003). Data of 30 children with complete behavioural, structural and functional datasets were used for model estimation. The area under the receiver operating characteristic curve (AUC) of the neural model was 0.86 (standard error (SE) = 0.07, 95% confidence interval (CI) = 0.72–1.00, d = 1.53). The AUC of the behavioural model was 0.76 (SE = 0.09, 95% CI = 0.58–0.94, d = 1.00), and the AUC of a combined model was 0.91 (SE = 0.05, 95% CI = 0.81–1.00, d = 1.90). Statistical comparison of AUCs showed that the combined model has significantly higher discrimination power than the behavioural model (D = −2.00, p = 0.0464, two-tailed), while there were no significant differences between AUCs of the neural model and the behavioural model (D = −0.95, p = 0.3429, two-tailed) and the combined model and the neural model (D = −0.91, p = 0.3644, two-tailed) (Fig. 2).

4. Discussion

Here, we combined psychometric testing with functional and structural MRI longitudinally assess children before they underwent literacy instruction and again at the end of second grade when we determined their literacy outcome. Our results provide converging evidence that participants with DD differ from typically developing individuals in terms of reduced phonological processing skills and increased gyrification of the left auditory cortex. Additionally, we found altered functional and structural connectivity within a left-hemispheric network including the auditory cortex, planum temporale, premotor cortex, and arcuate fasciculus.

We were able to replicate the consistently reported finding that phonological awareness most reliably distinguishes future dyslexic participants from controls before literacy instruction, followed by phonological short-term memory and rapid naming (Moll et al., 2014; Ziegler et al., 2010). Accordingly, our sample accurately represents the behavioural traits that are commonly observed in DD. Reports of older dyslexic children and adults lacking phonological impairments (Bosse et al., 2007; Peyrin et al., 2012) may be used to question the validity of phonological accounts of dyslexia. However, such accounts do not necessarily rule out that these individuals did show poor phonological skills early in life. In fact, typical performance in phonological tasks may indicate successful compensatory mechanisms that are in place during later stages of development, mitigating the behavioural deficits typically observed.

Somewhat unexpected, our behavioural assessment revealed that the groups did not significantly differ in terms of rapid naming, a measure prominently associated with reading outcome especially for transparent orthographies (de Jong and van der Leij, 2003). A possible reason for this discrepancy may be that a more comprehensive analysis of this ability includes rapid naming of letters in addition to colours of objects. However, it was not possible to perform a letter-based subtest in the current study since children were not able to read yet.

On the behavioural basis of phonological processing difficulties, we found neural signatures of dyslexia within a network of cortical areas that are known to support speech processing and are crucial for literacy learning. In particular, reduced functional connectivity between the primary auditory cortex and left planum temporale relates to a large body of literature highlighting the importance of these systems for spectro-temporal segmentation of the continuous speech stream into discrete sounds (Giraud and Poeppel, 2012; Lehongre et al., 2011). Thus, this difference in functional coherence might reflect a local core deficit of disrupted phonological processing. According to the rapid auditory processing deficit hypothesis of dyslexia, it can be expected that this deficit is most pronounced when processing speech sounds that are characterized by rapidly successive acoustic changes, such as the brief formant transitions (40 msec) that are the sole differentiating feature between syllables such as /ba/and /da/ (Tallal, 2004). This hypothesis needs to be tested in the future by making use of the high spatial and temporal resolution of magnetoencephalography.

Interestingly, the difference in functional connectivity between the auditory cortex and the planum temporale was only significant for male, but not female participants. A putative explanation for this observation based on rodent work might be that higher testosterone levels potentially lead to more pronounced differences in cortical development (Rosen et al., 1999), thus possibly impacting boys more severely than their female counterparts. Nevertheless, considering the substantially lower number of dyslexic girls compared to boys (n = 5 vs. n = 11) in this study, the result of this sex comparison needs to be interpreted with caution. Yet, a greater susceptibility to brain developmental disruptions for males might offer a first preliminary neural explanation for sex differences in reading skills reported across languages and educational practices (Stoet and Geary, 2013).

Further downstream, in a branch of the left arcuate fasciculus that connects the planum temporale with the ventral premotor cortex, we found significantly increased streamline density in DD versus controls. This measure was derived via tractography, modelling prominent fibre pathways via the number of streamlines passing a given voxel (Behrens et al., 2003). Thus, streamline density may be interpreted as an index of connectivity strength (Müller-Axth et al., 2017). In contrast, previous diffusion-weighted studies predominantly report reading-related differences within this tract in terms of fractional anisotropy. As fractional anisotropy is a rather unspecific measure that may be sensitive to a range of microstructural properties such as variance in fibre orientation or axonal diameter (Riffert et al., 2014), it is difficult to reconcile the current finding with reports of reduced fractional anisotropy in children at
familial risk of developing dyslexia (Kraft et al., 2016; Langer et al., 2017; Wang et al., 2017) and poor readers (Wang et al., 2017). Still, our finding of increased connectivity strength of the left arcuate fasciculus as assessed via streamline density is in line with a longstanding view that has been recently corroborated by meta-analyses of fMRI studies in dyslexic children. Namely, individuals with dyslexia hyperactivate the ventral premotor cortex during speech processing, presumably because they have to rely more strongly on articulation strategies to compensate for faulty encoding of phonemes in the planum temporale (Richlan et al., 2011; Shaywitz, 1998).

In the current study, we investigated the structure of white matter tracts in terms of FA, MD, and SLDF. Methodological advantages and limitations inherent to the use of these measures for assessing white matter structure are worth noting here. FA and MD are both based on the diffusion tensor, representing the major diffusion direction in a voxel. However, this model becomes unreliable for voxels containing multiple fiber bundles oriented in different directions or complex configurations (Behrens et al., 2007). Moreover, tensor-derived measures such as FA and MD may be further influenced by partial volume effects, as well as the degree of myelination, number and density of axonal projections crossing through the voxel under consideration (Alexander et al., 2001). Differences in terms of FA or MD might be driven by a combination of these aspects, impeding a precise interpretation of observed effects. SLD as analyzed here was derived from probabilistic tractography based on a multi-fiber model. While potentially producing some false positive connections (Knösche et al., 2015), probabilistic multi-fiber tracking is more sensitive to the distribution of connectivity included secondary and non-dominant fiber directions than deterministic approaches (Behrens et al., 2007). During each step of the tracking process itself, the respective next propagation direction is drawn from the probabilistic fiber orientation density function. Consequently, the likelihood of a streamline running through a specified voxel decreases with the distance of that voxel from the respective seed point, resulting in sparse and less reliable values at the tractogram’s edges. Finally, while streamline density measures show better test–retest performance compared to tensor-derived measures like FA (Buchanan et al., 2014), they are also modulated by sources of noise affecting the quality diffusion-weighted imaging more generally, such as the signal to noise ratio (Huang et al., 2004), subject motion or physiological noise (Bihan et al., 2006).

With MRI it is difficult to identify neurobiological sources of the structural auditory cortex alterations identified in our analysis. One possible explanation may be that the observed differences in gyriﬁcation capture faulty neural migration in the human temporal lobe (Giraud and Ramus, 2013). Typically, neural migration is a process that is completed before birth (Bystron et al., 2008), following a well-orchestrated cascade of cellular regulatory processes giving rise to the highly regular six-layered cortical architecture (Geschwind and Rakic, 2013). Importantly, ex vivo staining studies report neural migration anomalies such as mislocated neurons and exuberant numbers of small folds (i.e., heterotopias and polymicrogyria, respectively) located mainly in left peri-sylvian regions of dyslexic adults (Galaburda et al., 1985; Galaburda and Kemper, 1979). Such anomalies could lead to disruptions of the laminar cortical structure (Skrede et al., 2018a), potentially affecting further developmental trajectories of cortical thickness and gyriﬁcation, in addition to probable changes of local functional interactions in affected regions as described above. It should be noted, however, that the neuronal migration hypothesis of dyslexia needs additional experimental support beyond scale, indicating general work in rodents and case and pilot studies in humans (Guidi et al., 2018)

In line with a previous study that focused on cortical thickness (Clark et al., 2014), we identified structural alterations of the left primary auditory cortex as the only neural feature that persistently distinguished dyslexic participants from controls, not only before, but also two years after literacy instruction has begun. There can be many reasons for the discontinuity of the functional and structural connectivity effects from time point 1 to time point 2. For example, distinct maturational trajectories might lead to early differences that no longer reach signiﬁcance in the first school years (Yeatsman et al., 2012).

Increased gyriﬁcation stood out as the only signiﬁcantly different surface-based measure, while cortical thickness, cortical folding, and sulcus depth were similar between groups. The gyriﬁcation index employed in this study is inﬂuenced by changes in terms of magnitude and frequency of cortical folding (Liders et al., 2006). Interestingly, increased gyral frequency within the left Heschl’s gyrus has been previously described as an anatomical risk factor for phonological deﬁcits related to reading difﬁculties (Leonard et al., 2001; Leonard et al., 2006). While gyriﬁcation is associated with reduced short-range axonal distance, typically assumed to increase local neural efﬁciency (White et al., 2010), excessive gyriﬁcation may as well reﬂect anomalous neural organisation. Speciﬁcally, the gyriﬁcation index might capture the above-mentioned polymicrogyria that extend well beyond the millimetre scale and are thus large enough to be detected with common in vivo MRI resolution at 3 T (Galaburda et al., 2006).

DD is a deﬁcit that can affect several sensory and cognitive domains (Heim et al., 2008), which has led to an abundance of neural accounts of its developmental origins (Table 1). Nevertheless, the current study revealed differences between future dyslexic children and controls within a conﬁned network associated with speech sound processing. We were unable to replicate previously reported effects beyond phonological processing regions in the present sample (Table 1). It is clear that this insight has to be taken with the care generally devoted to null effects and calls for cautious interpretation. However, methodological discrepancies do not account for these negative results. Most importantly, given that the size of our sample was comparable or even superior to previous work (i.e., N equal or larger), it is unlikely that lack of replication simply reﬂects lack of statistical power in our study – although the sample size is still small.

Following accounts of auditory or visual sensory deﬁcits in dyslexia (Table 1), it is important to note that the current analysis did not reveal differences between dyslexic participants and controls in subcortical regions. It has to be acknowledged that functional analyses of subcortical regions prove particularly challenging due to increased levels of physiological noise originating from brainstem pulsation (Guimaraes et al., 1998). However, the nuisance regression algorithm applied to our data has been repeatedly shown to effectively remove false-negative activation across the whole brain including the thalamus and the brainstem, thus maximising the sensitivity of cortico-subcortical functional connectivity analyses (Behzadi et al., 2007; Ghisleni et al., 2015). Consequently, given our employed methodology, the observed null results in subcortical regions seem unlikely to be due to disruptions induced by physiological noise.

A prospective classiﬁcation model based on the three neural indices identiﬁed in our experiments signiﬁcantly predicted from the MRI data whether preliterate kindergarten children would develop dyslexia later in school. A similar behavioural model based on psychometric predictors did not reach signiﬁcance, but also did not perform signiﬁcantly worse than the neural model. Still, preliterate prediction of DD was signiﬁcantly improved when adding the brain measures to the behavioural model. The top performing combined model capturing phonological deﬁcits not only at the behavioural, but also at the neural level, showed excellent discriminatory power with an AUC of 0.91.

Previous longitudinal work on pre-literate children with familial risk of DD suggests a differentiation between structural and functional brain measures related to family risk versus actual reading outcomes. Speciﬁcally, basic auditory processing as assessed with ERPs (Hakvoort et al., 2015) and phoneme representations as assessed with multivariate fMRI responses (Vandermoten et al., 2019) were found to relate to family risk rather than later literacy performance. In contrast, the structure of the arcuate fasciculus was found to be related to reading outcomes but not to family risk (Vanderauwera et al., 2017). Disentangling the effects related to family risk versus reading outcome, however, was not the focus of the current study. Instead, the children in the dyslexic group were selected
purely based on reading outcome, and compared to control children that neither showed literacy deficits, nor exhibited a corresponding family history. Therefore, familial risk status was used as a covariate in all statistical models to minimise the variance induced by this factor.

The sample size of the current study is limited due to the effortful recruitment and time-consuming examination of a highly specific population of children. Hence, the results from this exploratory study await confirmation in larger follow-up studies guided by a priori analysis, as is the case for all other findings discussed here (Table 1). In addition, it remains to be shown in future work which functional and structural changes might occur later during development as a result of persistent deficits and reduced reading experience. Another limitation is that, despite the universal relevance of intact phonological processing for literacy learning, the present findings derived from a German sample might be to some degree specific to orthographies with relatively regular symbol-to-sound correspondences or alphabetic writing systems. Therefore, the current results must await generalization to less transparent and non-alphabetic writing systems in follow-up studies. Finally, for transparent orthographies like German, it has been shown that isolated reading and spelling disorders occur frequently (Fischbach et al., 2013; Landier and Moll, 2010). This phenomenon can be explained by the fact that the German orthography is relatively transparent with respect to reading (grapheme-to-phoneme mapping), but less transparent with respect to spelling (phoneme-to-grapheme mapping). Accordingly, a substantial proportion of German children with dyslexia reveal pronounced spelling difficulties while their reading skills still fall within the range of average performance (Wimmer and Mayringer, 2002). The characteristics of our sample are in line with this notion. There is an ongoing debate, however, on whether or not isolated disorders are based on the same or distinct deficits. While our study did not permit an in-depth analysis of potentially distinct neural profiles of isolated compared to combined deficits, this issue deserves more careful attention in future work.

In sum, our work adds to the understanding of the possible emergence of DD by unifying brain functional, brain structural and behavioural dimensions of a speech processing deficit that precedes literacy acquisition.

Author contributions

UK, ADF and MAS designed the study. IK, LD and MAS collected the data. UK and MAS analyzed the data. All authors interpreted the data. GS, NN and JB provided additional critical feedback on data interpretation. UK and MAS wrote the manuscript. UK designed the neuroimaging analysis and interpretations. NN and JB provided additional critical feedback on data interpretation. JB, JH, MC, LK, WC, SV, VL, DM, ED, VS, SSA, LYM, MB, CS, and ZO provided subject recruitment and data collection.

Data and code availability

The data and/or code used in the current study are available from the corresponding author upon reasonable request.

Declaration of competing interest

None.

Acknowledgements

This work was supported by the Max Planck Society and the Fraunhofer Society (Grant number M.E.A.NEPF0001). We thank A. Geb-Sandmann for help on figure preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2020.116633.

References


Bosron, M.L., Tainturier, M.J., Valdois, S., 2007. Developmental dyslexia: the visual information in larger follow-up studies guided by a priori analysis, as is the case for all other findings discussed here (Table 1). In addition, it remains to be shown in future work which functional and structural changes might occur later during development as a result of persistent deficits and reduced reading experience. Another limitation is that, despite the universal relevance of intact phonological processing for literacy learning, the current results must await generalization to less transparent orthographies like German, it has been shown that isolated reading and spelling disorders occur frequently (Fischbach et al., 2013; Landier and Moll, 2010). This phenomenon can be explained by the fact that the German orthography is relatively transparent with respect to reading (grapheme-to-phoneme mapping), but less transparent with respect to spelling (phoneme-to-grapheme mapping). Accordingly, a substantial proportion of German children with dyslexia reveal pronounced spelling difficulties while their reading skills still fall within the range of average performance (Wimmer and Mayringer, 2002). The characteristics of our sample are in line with this notion. There is an ongoing debate, however, on whether or not isolated disorders are based on the same or distinct deficits. While our study did not permit an in-depth analysis of potentially distinct neural profiles of isolated compared to combined deficits, this issue deserves more careful attention in future work.

In sum, our work adds to the understanding of the possible emergence of DD by unifying brain functional, brain structural and behavioural dimensions of a speech processing deficit that precedes literacy acquisition.

Author contributions

UK, ADF and MAS designed the study. IK, LD and MAS collected the data. UK and MAS analyzed the data. All authors interpreted the data. GS, NN and JB provided additional critical feedback on data interpretation. UK and MAS wrote the manuscript. UK designed the figures. All authors critically reviewed and approved the manuscript.

Data and code availability

The data and/or code used in the current study are available from the corresponding author upon reasonable request.

Declaration of competing interest

None.

Acknowledgements

This work was supported by the Max Planck Society and the Fraunhofer Society (Grant number M.E.A.NEPF0001). We thank A. Geb-Sandmann for help on figure preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2020.116633.
https://doi.org/10.1016/j.neuroimage.2014.01.002.
https://doi.org/10.1056/NEJM199801293380507.
https://doi.org/10.1212/WNL.0000000000004931.
https://doi.org/10.1111/desc.12680.
https://doi.org/10.1126/sciadv.1602612.
https://doi.org/10.1371/journal.pone.0057988.
https://doi.org/10.1038/nrn1499.
https://doi.org/10.1111/desc.12045.
https://doi.org/10.1016/j.dcn.2017.08.005.
https://doi.org/10.1111/desc.12857.
https://doi.org/10.1111/desc.12857.
https://doi.org/10.1093/cercor/bhw095.
https://doi.org/10.1093/cercor/bhx001.
https://doi.org/10.1037//0022-0663.94.2.272.
https://doi.org/10.1073/pnas.1206792109.